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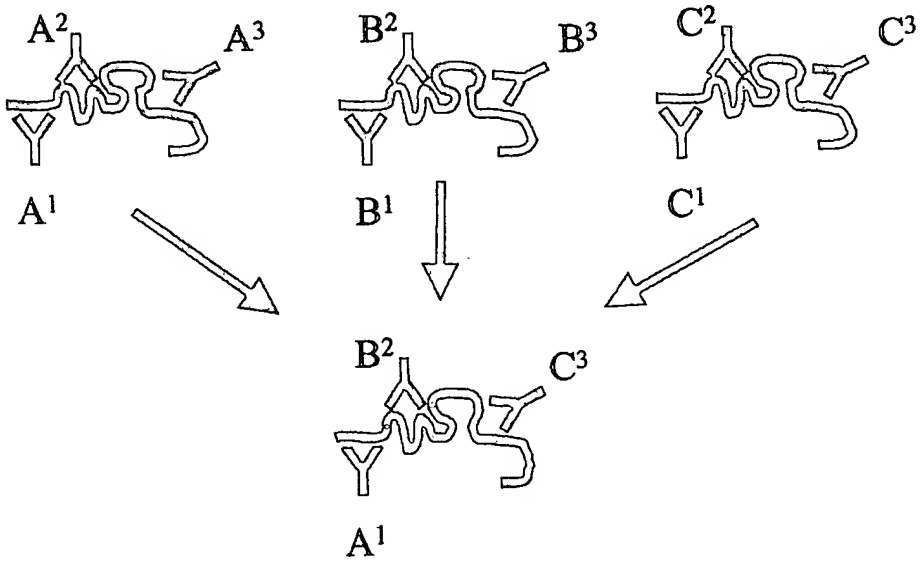

(57) Abstract

This invention is directed to antigen library immunization, which provides methods for obtaining antigens having improved properties for therapeutic and other uses. The methods are useful for obtaining improved antigens that can induce an immune response against pathogens, cancer, and other conditions, as well as antigens that are effective in modulating allergy, inflammatory and autoimmune diseases.

WHAT IS CLAIMED IS

1. An recombinant multivalent antigenic polypeptide that comprises a first antigenic determinant of a first polypeptide and at least a second antigenic determinant from a second polypeptide.

2. The multivalent antigenic polypeptide of claim 1, wherein the polypeptide comprises at least a third antigenic determinant from a third polypeptide.

3. The multivalent antigenic polypeptide of claim 1, wherein the first and second polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens associated with inflammatory conditions, antigens associated with allergic reactions, and antigens from infectious agents.

4. The multivalent antigenic polypeptide of claim 3, wherein the antigens are from a virus, a parasite, or a bacteria.

5. The multivalent antigenic polypeptide of claim 4, wherein the antigens are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a Dengue virus, a Hanta virus, an HIV, a hepatitis B virus, a hepatitis C virus, and a *Herpes simplex* virus.

6. The multivalent antigenic polypeptide of claim 5, wherein the antigens are envelope proteins.

7. The multivalent antigenic polypeptide of claim 4, wherein the antigens are from a bacteria and are selected from the group consisting of a Yersinia V antigen, a *Staphylococcus aureus* enterotoxin, a *Streptococcus pyogenes* enterotoxin, a *Vibrio cholera* toxin, an enterotoxigenic *Escherichia coli* heat labile enterotoxin, a OspA and a OspC polypeptide from a *Borrelia* species, an Antigen 85 polypeptide from a *Mycobacterium* species, a VacA and a CagA polypeptide from *Helicobacter pylori*, and an MSP antigen from *Plasmodium falciparum*.

8. The multivalent antigenic polypeptide of claim 1, wherein the multivalent antigenic polypeptide exhibits reduced affinity to IgE from a mammal compared to the first or second polypeptides.

9. The multivalent antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different serotypes of a pathogenic organism.

10. The multivalent antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different species of pathogenic organism.

11. The multivalent antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are allergens.

12. The multivalent antigenic polypeptide of claim 11, wherein the allergens are dust mite allergens, grass pollen allergens, birch pollen allergens, ragweed pollen allergens, hazel pollen allergens, cockroach allergens, rice allergens, olive tree pollen allergens, fungal allergens, mustard allergens, and bee venom.

13. The multivalent antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are associated with an inflammatory or autoimmune disease.

14. The multivalent antigenic polypeptide of claim 13, wherein the first polypeptide and the second polypeptide are autoantigens associated with a disease selected from the group consisting of multiple sclerosis, scleroderma, systemic sclerosis, systemic lupus erythematosus, hepatic autoimmune disorder, skin autoimmune disorder, insulin-dependent diabetes mellitus, thyroid autoimmune disorder, and rheumatoid arthritis.

15. The multivalent antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are cancer antigens or sperm antigens.

16. A recombinant antigen library comprising recombinant nucleic acids that encode antigenic polypeptides, wherein the library is obtained by recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes a disease-associated antigenic polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids.

17. The recombinant antigen library of claim 16, wherein the first and second polypeptides are toxins.

18. A method of obtaining a polynucleotide that encodes a recombinant antigen having improved ability to induce an immune response to a disease condition, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes an antigenic polypeptide that is associated with the disease condition, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and

(2) screening the library to identify at least one optimized recombinant nucleic acid that encodes an optimized recombinant antigenic polypeptide that has improved ability to induce an immune response to the disease condition.

19. The method of claim 18, wherein the method further comprises:

(3) recombining at least one optimized recombinant nucleic acid with a further form of the nucleic acid, which is the same or different from the first and second forms, to produce a further library of recombinant nucleic acids;

(4) screening the further library to identify at least one further optimized recombinant nucleic acid that encodes a polypeptide that has improved ability to induce an immune response to the disease condition; and

(5) repeating (3) and (4), as necessary, until the further optimized recombinant nucleic acid encodes a polypeptide that has improved ability to induce an immune response to the disease condition.

20. The method of claim 18, wherein the disease-associated polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens associated with inflammatory conditions, antigens associated with allergic reactions, and antigens associated with infectious agents.

21. The method of claim 18, wherein the disease condition is an infectious disease and the first and second forms of the nucleic acid each encode an antigen of a different serotype of a pathogenic agent.

22. The method of claim 21, wherein the first and second forms of the nucleic acid are each from a different species of pathogen.

23. The method of claim 21, wherein the screening is accomplished by:

introducing into a test animal either:

a) the library of recombinant nucleic acids, or

b) recombinant polypeptides encoded by the library of recombinant nucleic acids;

introducing the pathogenic agent into the test animal; and

determining whether the test animal is resistant to challenge by the pathogenic agent.

24. The method of claim 23, wherein the pathogenic agent introduced into the test animal is of a different serotype than that used as a source of the first and second forms of the nucleic acid.

25. The method of claim 23, wherein the library is subdivided into a plurality of pools, each of which pools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

26. The method of claim 25, wherein the pools that include an optimized recombinant nucleic acid are further subdivided into a plurality of subpools, each of which

subpools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

27. The method of claim 18, wherein the optimized recombinant nucleic acid encodes a multivalent antigenic polypeptide and the screening is accomplished by:

expressing the library of recombinant nucleic acids in a phage display expression vector such that the recombinant antigen is expressed as a fusion protein with a phage polypeptide that is displayed on a phage particle surface;

contacting the phage with a first antibody that is specific for a first serotype of the pathogenic agent and selecting those phage that bind to the first antibody;

contacting those phage that bind to the first antibody with a second antibody that is specific for a second serotype of the pathogenic agent and selecting those phage that bind to the second antibody;

wherein those phage that bind to the first antibody and the second antibody express a multivalent antigenic polypeptide.

28. The method of claim 27, wherein the screening further comprises contacting those phage that bind to the first and second antibodies with one or more additional antibodies, each of which is specific for an additional serotype of the pathogenic agent, and selecting those phage that bind to the respective additional antibodies.

29. The method of claim 27, wherein the phage display expression vector comprises a suppressible stop codon between the recombinant nucleic acid and the phage polypeptide, whereby expression in a host cell which comprises a corresponding suppressor tRNA results in production of the fusion protein and expression in a host cell which lacks a corresponding suppressor tRNA results in production of the recombinant antigen not as a fusion protein.

30. The method of claim 18, wherein the optimized recombinant antigen exhibits an enhanced expression level in a host cell and the screening is accomplished by expression of each recombinant nucleic acid in the host cell and subjecting the host cells to

flow cytometry-based cell sorting to obtain those host cells that display the recombinant antigen on the host cell surface.

31. The method of claim 18, wherein the improved property is selected from the group consisting of:

improved immunogenicity;

enhanced cross-reactivity against different forms of the disease-associated antigenic polypeptide;

reduced toxicity;

improved adjuvant activity *in vivo*; and

improved production of the immunogenic polypeptide.

32. The method of claim 31, wherein the improved property is enhanced cross-reactivity against different forms of the disease-associated polypeptide and the first and second forms of the nucleic acid are from a first and a second form of the disease-associated polypeptide.

33. The method of claim 32, wherein the first and second forms of the disease-associated polypeptide are obtained from at least a first and second species of a pathogenic agent and the optimized recombinant nucleic acid encodes a recombinant polypeptide that induces a protective response against both species of the pathogenic agent.

34. The method of claim 33, wherein the recombinant polypeptide induces a protective response against at least one additional species of the pathogenic agent.

35. The method of claim 33, wherein the pathogenic agent is a toxin.

36. The method of claim 33, wherein the pathogenic agent is a virus or a cell.

37. The method of claim 33, wherein the disease-associated polypeptide is a *Yersinia* V-antigen.

38. The method of claim 37, wherein the at least first and second forms of a nucleic acid are obtained from at least a first and second species of *Yersinia*.

39. The method of claim 38, wherein the *Yersinia* species are selected from the group consisting of *Y. pestis, Y. enterocolitica,* and *Y. pseudotuberculosis*.

40. The method of claim 33, wherein the pathogenic agent is a bacterial toxin.

41. The method of claim 18, wherein the disease condition is cancer and the screening step involves introducing the optimized recombinant nucleic acids into a genetic vaccine vector and testing library members for ability to inhibit proliferation of cancer cells or inducing death of cancer cells.

42. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a tumor specific antigen.

43. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a molecule which is capable of inhibiting proliferation of cancer cells.

44. The method of claim 18, wherein the disease condition is an inflammatory response which has an unknown or no antigen specificity and the screening step involves one or more of the following:

a) determining the ability of the genetic vaccine vector to induce cytokine production by PBMC, synovial fluid cells, purified T cells, monocytes/macrophages, dendritic cells, or T cell clones;

b) determining the ability of the genetic vaccine vector to induce T cell activation or proliferation; and

c) determining the ability of the genetic vaccine vector to induce T cell differentiation to T_H1 or T_H2 cells.

45. The method of claim 18, wherein the disease condition is an autoimmune response.

46. The method of claim 45, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a T_H1-mediated response to a T_H2-mediated response.

47. The method of claim 18, wherein the disease condition is an allergic immune response.

48. The method of claim 47, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a T_H2-mediated response to a T_H1-mediated response.

49. The method of claim 47, wherein the optimized recombinant antigenic polypeptide induces an immune response characterized by predominant IgG and IgM expression and reduced IgE expression.

50. The method of claim 47, wherein the optimized recombinant antigenic polypeptide is not recognized by pre-existing IgE molecules present in sera of atopic mammals.

51. The method of claim 50, wherein the optimized recombinant antigenic polypeptide retains T cell epitopes that are involved in modulating a T cell response.

52. A method of obtaining a recombinant viral vector which has an enhanced ability to induce an antiviral response in a cell, the method comprising the steps of:

(1) recombining at least first and second forms of a nucleic acid which comprise a viral vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant viral vectors;

(2) transfecting the library of recombinant viral vectors into a population of mammalian cells;

(3) staining the cells for the presence of Mx protein; and

(4) isolating recombinant viral vectors from cells which stain positive for Mx protein, wherein recombinant viral vectors from positive staining cells exhibit enhanced ability to induce an antiviral response.

53. The method of claim 52, wherein the viral vector comprises an influenza viral genomic nucleic acid.

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